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CASE REPORTS

Molecular Evidence of Perinatal Transmission of *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* to a Child[▽]

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***Bartonella vinsonii* subsp. *berkhoffii*, *Bartonella henselae*, or DNA of both organisms was amplified and sequenced from blood, enrichment blood cultures, or autopsy tissues from four family members. Historical and microbiological results support perinatal transmission of *Bartonella* species in this family. It is of clinical relevance that *Bartonella* spp. may adversely influence human reproductive performance.**

CASE REPORT

At the time of *Bartonella* testing, the father, mother, and son were 47, 46, and 10 years of age, respectively. Prior to meeting in 1989, both parents were reportedly healthy. The father and mother were born and raised on Long Island, where the father was a carpenter and the mother a graphic designer. As children and teenagers, the father had daily contact with dogs and the mother had daily contact with cats and dogs; however, both parents reported minimal exposure to other animals or arthropod vectors. At 16 years of age, the mother was severely bitten and scratched multiple times while bathing a feral, flea-infested cat. The couple was married in 1991 and their twins, conceived following *in vitro* fertilization, were born by cesarean section in 1998. The twin sister died nine days after birth, due to hypoplastic left heart syndrome. The family relocated to Florida in 2000, when their son was 15 months old. After their marriage, the couple did not maintain a pet cat and did not acquire a dog until the summer of 2009.

Waxing and waning symptoms, primarily consisting of fatigue, headaches, and urogenital pain and straining while voiding developed in both parents shortly after they were married. In 1991, the mother had a cervical biopsy due to vaginal warts. Between 1992 and 1995, the mother was diagnosed with irritable bowel syndrome, interstitial cystitis, and infertility. By 1999, both parents were experiencing persistent symptoms, including frequent headaches, memory loss and confusion, irritability, insomnia, bladder dysfunction, and balance problems. Between 2000 and 2009, all three family members reported intermittent joint pain, muscle pain, restlessness, shortness of breath, episodic tachycardia, rashes, rectal puritis, hypersensitivity to various foods, fatigue, episodes of extreme anxiety and irritability, cognitive impairment, and stabbing urethral pain when voiding, attributed to candidiasis. The husband, a nonsmoker, also had a chronic cough for years and occasional urinary tract infections.

Family members had sought care from primary care physicians and several specialists, including an internist, gastroenterologist, urologist, infectious disease physician, neurotoxin expert, and several naturopaths. Based upon blood mercury results, both parents had dental fillings removed in 2001. During the 4 years prior to *Bartonella* testing, the family had been treated with cholestyramine for suspected toxic mold exposure in their home in Florida. Neither this treatment, removal of the dental fillings, nor thorough remediation of the household environment while in Florida resulted in symptomatic improvement. The husband and wife had also been treated with doxycycline, azithromycin, and ciprofloxacin for *Chlamydia pneumoniae* and *Mycoplasma pneumonia* and with nitazoxanide for *Blastocystis hominis*. None of these treatments elicited symptomatic improvement. Beginning in 2000, both parents were repeatedly treated with long courses (up to 1 to 3 years) of fluconazole and nystatin for oral or vaginal candidiasis, with improvement in urogenital symptoms coinciding with each treatment. For financial and medical reasons, the family moved back to New York in 2009. There was no familial history of irritable bowel syndrome, interstitial cystitis, infertility, or congenital heart defects. Despite three additional attempts at *in vitro* fertilization, the mother did not become pregnant again.

The son had severe colic as a baby and subsequently experienced frequent night walking, night sweats, hyperactivity and irritability, dark circles under his eyes, frequent ear infections and eye blinking. The son was tested for mercury toxicity, *Mycoplasma*, *Chlamydia*, and intestinal parasites and all test results were negative. He had infrequent to no contact with animals or arthropod vectors. Despite his medical problems, the son attended school and participated in age-appropriate activities.

In January 2009, the primary author was contacted by the mother, requesting *Bartonella* testing as a component of an

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TABLE 1. Serological, blood culture, and 16S-23S intergenic spacer PCR test results for a family in which members were infected with *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae*[a]

Patient, type of sample, date (mo/day/yr)	*Bartonella* IFA reciprocal titer for:				PCR/DNA sequencing result(s) for:		
	Bvb I	Bvb II	Bvb III	Bh	Direct extraction	BAPGM enrichment culture	Subculture
Father							
Blood, 4/11/2009	<16	<16	<16	<16	Neg	Bvb II	Neg
Mother							
Blood, 4/11/2009	<16	<16	<16	<16	Bh SA2	Bvb II	Neg
Placenta, 1998	NA	NA	NA	NA	Neg	NA	NA
Cervical biopsy specimen, 1991	NA	NA	NA	NA	Bh SA2	NA	NA
Son							
Blood, 4/11/2009	16	64	16	64	Neg	Neg	Neg
Blood, 6/10/2009	64	1,024	256	1,024	Bh SA2[b]	Bh/Bvb[b]	Neg
Blood, 9/9/2009	256	128	64	256	Neg	Bh[b]	Neg
Daughter							
Brain, 1998	NA	NA	NA	NA	Bh SA2	NA	NA
Liver, 1998	NA	NA	NA	NA	Bh SA2/Bvb II	NA	NA
Spleen, 1998	NA	NA	NA	NA	Neg	NA	NA

[a] Bvb I, II, and III, *B. vinsonii* subsp. *berkhoffii* genotypes I, II, and III; Bh SA2, *B. henselae* strain SA2; NA, serology and culture not applicable (PCR testing was performed on stored paraffin-embedded tissues); Neg, negative.

[b] These ITS PCR results were confirmed by targeting the *rpoB* gene, as overlapping sequences were obtained following amplification of the 16S-23S intergenic spacer region. The *rpoB* gene does not allow for *Bartonella* spp. strain discrimination; however, the partial, nonoverlapping ITS sequence confirmed an SA2 strain. All PCR results were confirmed by DNA sequencing.

IRB-approved study (North Carolina State University Institutional Review Board, IRB number 164-08-05). Testing for the father, mother, and son was performed using a previously described diagnostic platform that incorporates enrichment culture of patient blood samples in *Bartonella* alphaproteobacterial growth medium (BAPGM) (6, 7, 12, 24). In addition to *Bartonella* spp., this nonselective medium will isolate other common and fastidious bacterial species (8). Previously described 16S-23S intergenic spacer (ITS) region and polymerase beta subunit (*rpoB*) gene PCR assays were used for the detection of *Bartonella* sp. DNA in this study (11, 22). Four to six independent 16S-23S ITS PCRs were performed for each patient sample set. Specifically, DNA was extracted directly from EDTA anticoagulated blood and from serum samples. Simultaneously, 2 ml of the aseptically obtained blood was inoculated into 10 ml of BAPGM as an enrichment culture step. Following incubation for 7 to 14 days in liquid culture medium, DNA was extracted for PCR testing. Subcultures on blood agar were incubated in 5% CO_2 for up to 5 weeks. Archived paraffin-embedded postmortem tissues from the female twin and cervical and placental biopsy tissues from the mother were obtained for PCR testing and DNA sequencing. Each tissue was processed at three different time points, with extreme care taken to avoid DNA carryover or PCR amplicon contamination (29). Our laboratory took several precautionary approaches to minimize and to potentially eliminate the risk of PCR contamination and to prevent the carryover of either genomic or preamplified PCR products between samples. (i) Each component of sample processing (DNA extraction, PCR preparation, PCR amplification, and gel analysis) was performed in three physically separated rooms. A unidirectional work flow was enforced whereby any person handling materials or solutions in the PCR amplification and gel analysis room was not allowed to return to or perform work in the other two rooms. (ii) Positive controls consisting of 0.001 picograms per microliter of *Bartonella* genomic DNA (equivalent to 0.5 genome copies per microliter) were used for all PCR runs. This represents only twice the amount of template DNA that can be amplified by our protocols. A low-DNA-concentration control was purposely used in order to assess PCR performance and to minimize the potential for DNA carryover from the positive control. (iii) A negative control, consisting of *Bartonella*-free host DNA, was tested with every PCR run. To further ensure that any DNA template carryover from the positive-control sample would be detected, the negative control was prepared and allocated just after the positive-control sample and before any patient samples were processed in each PCR run. During the course of this study, a Houston 1-ITS strain of *Bartonella henselae* was exclusively used as the positive control. No contamination was detected in any of the 300 negative-control samples processed during the year in which the patient samples in this study were tested.

Serology was performed using modifications of a previously described indirect fluorescent antibody test (10). *Bartonella vinsonii* subsp. *berkhoffii* and *B. henselae* antibodies were determined following traditional immunofluorescence antibody assay (IFA) practices with fluorescein-conjugated goat anti-human IgG and *B. vinsonii* subsp. *berkhoffii* genotypes I, II, and III and *B. henselae* (Houston I strain) antigens. Sera were screened at dilutions of 1:16 to 1:256, and positives were further tested with 2-fold dilutions out to a final dilution of 1:8,192.

B. henselae (SA2 strain) DNA was amplified and sequenced from the mother's serum sample, and *B. vinsonii* subsp. *berkhoffii* genotype II DNA was amplified and sequenced from the enrichment blood cultures from the father (day 14 culture) and the mother (day 7 and 14 cultures) (Table 1). The son's initial BAPGM platform results were negative. Because azithromycin

was administered for a sinus infection 1 week prior to obtaining blood for culture, the son was again tested for *Bartonella* bacteremia 8 and 20 weeks later (Table 1). ITS PCR results for the son's 8-week blood and serum samples were positive; however, direct sequencing generated partially overlapping sequences from each sample, with the nonoverlapping sequence consistent with *B. henselae* (SA2 strain). After ITS cloning, only *B. henselae* (GenBank accession number HMO42284) DNA was sequenced from the blood sample. However, using *rpoB* primers, followed by cloning, *B. vinsonii* subsp. *berkhoffii* (GenBank accession number accession number HMO42287) DNA was amplified and sequenced from the son's serum sample and from the enrichment blood culture (GenBank accession number HMO42288) (GenBank accession number HMO42284). Repeat testing, at 20 weeks, resulted in sequencing *B. henselae* DNA from the BAPGM enrichment culture. The father and mother were not seroreactive to any of the four *Bartonella* spp. test antigens, whereas the son was seroreactive to *B. vinsonii* subsp. *berkhoffii* genotypes I, II, and III and *B. henselae* antigens (Table 1). The family's pet dog, a 5-month-old male mixed breed acquired in 2009, was seronegative and PCR negative for all components of the BAPGM platform. All uninoculated BAPGM culture and PCR negative controls remained negative throughout the study.

Bartonella vinsonii subsp. *berkhoffii* genotype II (GenBank accession number HMO42286) and *B. henselae* (SA2 strain) DNA were amplified and sequenced from paraffin-embedded liver tissue and *B. henselae* (SA2 strain) DNA was sequenced from brain tissue (GenBank accession number HMO42285) collected during autopsy from the twin daughter, who died in 1998, nine days after birth. *B. henselae* (ITS SA2 strain) was also sequenced from the mother's cervical biopsy tissue obtained in 1991. The daughter's splenic and mother's placental tissues were PCR negative.

Members of the genus *Bartonella* are facultative intracellular bacteria belonging to the alpha 2 subgroup of the *Proteobacteria* (9). Prior to 1990, only two pathogenic *Bartonella* species, *B. bacilliformis* and *B. quintana*, were known to exist. Since 1990, more than 22 *Bartonella* species have been described, of which at least half have been implicated or confirmed as human pathogens (9, 13, 16, 26). An increasing number of arthropods, including biting flies, fleas, keds, lice, sandflies, and ticks, are confirmed or suspected vectors for *Bartonella* transmission among animal populations (2). On an evolutionary basis, these highly fastidious, Gram-negative bacteria have become adapted to one or more mammalian reservoir hosts, within which *Bartonella* spp. usually cause a long-lasting endotheliotropic infection accompanied by a relapsing intraerythrocytic bacteremia (9). Reservoir hosts for *Bartonella vinsonii* subsp. *berkhoffii* include dogs, coyotes, and foxes (4, 9, 23), and genotype II has been the most frequent strain found in blood samples from dogs and people in the United States (6, 7, 12, 14, 18). Cats are considered the primary reservoir host for *B. henselae*, and cat fleas (*Ctenocephalides felis*) facilitate vector transmission (9, 13, 16).

PCR amplification of *B. vinsonii* subsp. *berkhoffii* and *B. henselae* DNA from the female twin that died 9 days after cesarean section supports perinatal or *in utero* transmission of two *Bartonella* spp. and indicates that the mother was most likely infected with one or both bacteria prior to or during pregnancy. The twin brother was also coinfected with *B. vinsonii* subsp. *berkhoffii* and *B. henselae*. However, *rpoB* sequences do not distinguish among *B. vinsonii* subsp. *berkhoffii* genotypes, and efforts to define the genotype by sequencing *Bartonella* 16S-23S amplicons were not successful. As the mother's and daughter's samples contained the same *B. vinsonii* subsp. *berkhoffii* genotype and the same *B. henselae* ITS strain (SA2), it is plausible that both twins were infected *in utero*, after which the son sustained a persistent infection for 10 years. As the same *B. henselae* ITS SA2 strain type was also sequenced from the mother's 1991 cervical biopsy, the daughter's 1998 postmortem brain and liver tissues, and the mother's 2009 blood sample, it seems likely that this woman was infected with *B. henselae* for at least 18 years. If transmission of *B. henselae* to the mother occurred via bites and scratches that were sustained at 16 years of age while attempting to bathe a flea-infested feral cat, then this woman may have been persistently infected with *B. henselae* for 30 years.

Prior to her death, the daughter received several blood transfusions, which theoretically could represent an alternative source of *Bartonella* spp. transmission. *B. henselae* has been successfully isolated following experimental inoculation of blood units and after storage at 4°C for 35 days (20). Infection with a *Bartonella* sp. was implicated in the case of an aplastic anemia patient who developed shock and died after receiving several red blood cell transfusions (21). Also, following multiple blood transfusions, an 86-year-old hospitalized man seroconverted to *B. vinsonii* subsp. *berkhoffii* antigens and *B. vinsonii* subsp. *berkhoffii* genotype II was amplified and sequenced from a BAPGM blood culture obtained 4 days prior to his death (5).

To date, there have been a limited number of studies that address the potential impact of intravascular infection with a *Bartonella* sp. on reproductive performance. The parents of these children had attempted to conceive children for several years prior to resorting to *in vitro* fertilization. In addition, after the birth of their twins, several subsequent attempts at *in vitro* fertilization were not successful. In 1998, Kosoy and colleagues reported the isolation of *Bartonella* spp. from the embryos and neonates of naturally infected cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) (19). Subsequently, experimental transplacental transmission of *Bartonella birtlesii* to mice was accompanied by an increase in fetal deaths, increased fetal resorption, and low birth weights (3). In addition, female mice had a significantly higher *B. birtlesii* bacteremia than male mice, and pregnant mice had a higher level of bacteremia than nonpregnant control mice. Experimental inoculation of *B. henselae* to adult female cats was accompanied by decreased conception or failure to maintain pregnancy (15). In a study from France, *Bartonella bovis* bacteremia was significantly higher in pregnant cows than in nonpregnant cows, and the level of bacteremia rose during the last two-thirds of the gestation period (25). Collectively, observations from mice, cats, and cows indicate that the hormonal changes accompanying pregnancy may influence the severity of *Bartonella* spp. bacteremia following experimental or natural infection with a *Bartonella* sp. Recently, *B. henselae*

was demonstrated by immunohistochemistry, transmission electron microscopy, PCR, and DNA sequencing in an aborted equine fetus, further supporting a role for this genus in reproductive disorders (17). Structural differences in placentation may determine whether a *Bartonella* sp. can be transmitted from a mother to her offspring *in utero*; however, transplacental transmission may not be required to induce alterations in reproductive performance. Efforts to amplify *Bartonella* spp. DNA from the mother's paraffin-embedded placental tissue were not successful; therefore, perinatal transmission to both twins during the cesarean section rather than *in utero* transmission remains possible. Alternatively, DNA may have been denatured during the formalin fixation or storage process, thereby negating successful amplification of *Bartonella* spp. DNA. Whether the placenta in the latter stages of pregnancy could serve as a site for intense replication of *Bartonella* spp., as occurs with *Brucella*, a closely related alphaproteobacterium, has not been determined in any animal species (31).

As both parents were infected with the same *B. vinsonii* subspecies and genotype, the question arises as to when and how each became infected. Cat scratch disease, caused by *B. henselae*, is the best-documented example of direct animal-to-human transmission of a *Bartonella* species by scratch or bite inoculation (9). However, it is likely that several other *Bartonella* spp. can be transmitted from a chronically infected reservoir host to a human via a bite or scratch. For example, *Bartonella alsatica* transmission from wild rabbits to humans, presumably occurring during hunting and butchering processes, has been reported in patients with endocarditis or lymphadenitis in France (1, 27). Dog bite transmission of a *B. vinsonii* subspecies to a human was recently implicated, based upon serological evidence (28). As healthy dogs can be persistently bacteremic with *B. vinsonii* subsp. *berkhoffii* genotype II for at least 16 months, dogs and, more often, foxes or coyotes can serve as persistently infected reservoir hosts for this bacterium (9, 18). Recently, we reported *B. vinsonii* subsp. *berkhoffii* genotype II infection in a cat with recurrent osteomyelitis that spanned an 18-month time period, suggesting that a *B. vinsonii* subsp. *berkhoffii*-infected cat might be a source for human infection (30). Based upon the mother's history, it is possible that she became coinfected with *B. vinsonii* subsp. *berkhoffii* genotype II and *B. henselae* when caring for a flea-infested feral cat in 1978. Although we were only able to amplify *B. henselae* DNA from the mother's archived cervical tissues, we have previously reported preferential amplification of *B. henselae* DNA during *in vitro* studies in which BAPGM was inoculated with both *B. henselae* and *B. vinsonii* subsp. *berkhoffii* (24). Although it is increasingly obvious that dogs, humans, and other animal species can be coinfected with more than one *Bartonella* spp., molecular documentation of coinfection remains challenging and technical limitations may have contributed to our failure to document infection or coinfection with *B. vinsonii* subsp. *berkhoffii* and *B. henselae* in specific samples from these family members. As children, both parents had daily exposure to dogs, but they reported only infrequent contact with cats or dogs after their marriage.

Confirming infection with a *Bartonella* sp. remains extremely challenging. Bacterial isolation, serology, and PCR amplification of *Bartonella* spp. DNA directly from patient samples each have substantial diagnostic limitations (6, 7, 13, 16, 30). Enrichment culture using BAPGM was required to document infection with *B. vinsonii* subsp. *berkhoffii* in the blood samples from all three family members, whereas *B. henselae* DNA was successfully sequenced directly from the daughter's tissues and from the mother's and son's serum samples. Based upon the BAPGM platform results derived for this family, *B. henselae* was more likely to be detected following direct extraction from a blood or serum sample, whereas *B. vinsonii* subsp. *berkhoffii* was more likely to be detected only after enrichment culture. Based upon previously published studies describing the detection of *Bartonella* spp. using the BAPGM platform, it is necessary to test blood, serum, enrichment culture, and subcultures to achieve maximal detection of these bacteria in patient samples (5–8, 12). Administration of azithromycin to the son 1 week prior to the initial blood culture most likely contributed to the negative *Bartonella* PCR results, as coinfection was documented in a sample obtained 2 months later. As reported in previous studies, the mother and father did not have detectable antibodies to any of the four *Bartonella* spp. test antigens (6, 7). In contrast, the son was variably reactive to all three *B. vinsonii* subsp. *berkhoffii* genotypes and to *B. henselae* antigens in three different serum samples collected over a 20-week period. Although unproven, it appears that chronic intravascular infection with a *Bartonella* spp. may induce a degree of immunological anergy, resulting in undetectable levels of organism-specific antibodies in some chronically infected patients.

This study provides the first molecular microbiologic evidence that *B. vinsoniii* subsp. *berkhoffii* and *B. henselae* can be transmitted to children *in utero* or during cesarean section. Due to the availability of stored paraffin-embedded tissues from the mother, we were able to confirm infection with *B. henselae* in the mother 10 years before *in utero* or perinatal transmission of these bacteria to her children. Unfortunately, it was not possible to determine if *B. vinsonii* subsp. *berkhoffii* was transmitted horizontally between the parents after marriage or whether each parent independently acquired their infections through animal or vector contact.

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In conjunction with Sushama Sontakke and North Carolina State University, E. B. Breitschwerdt holds U.S. patent 7,115,385, Media and methods for cultivation of microorganisms, which was issued 3 October 2006. He is the chief scientific officer for Galaxy Diagnostics, a newly formed company that provides diagnostic testing for the detection of *Bartonella* species infection in animals and in human patient samples. Ricardo Maggi is the scientific technical advisor and laboratory director for Galaxy Dx.

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